



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Application No.: 09/831,954

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For: ESTROGENIC ESTRA-1,3,5(10)-TRIENES WITH  
DIFFERENTIAL EFFECTS ON THE ALPHA AND BETA  
ESTROGEN RECEPTORS, HAVING A LINEAR HYDROCARBON  
CHAIN FROM 5-9 CARBON ATOMS AT POSITION 11.

DECLARATION UNDER 37 C.F.R. §1.132

I, Antwan G. H. Ederveen declare as follows:

I am a pharmacologist, presently employed by N.V. Organon in the Netherlands as Section Head in the Pharmacology Department.

I was formerly a member of a lead optimization team, involved in pre-clinical research to find new estrogenic compounds for medical use.

I am familiar with the contents of the patent application for which this declaration is submitted to the US Patent Office and I am familiar also with the contents of Lobaccaro, C. et al., 'Steroidal Affinity Labels of the Estrogen Receptor 3. Estradiol 11- $\beta$ -n-Alkyl Derivatives Bearing A Terminal

Electrophilic Group: Anti-Estrogenic and Cytotoxic Properties' *Journal of Medicinal Chemistry*, vol. 40, no. 14, July 4, 1997, pages 2217-2227 and Napolitano E. et al., '11- $\beta$ -Substituted Estradiol Derivatives 2. Potential Carbon-11- and Iodine Labeled Probes for the Estrogen Receptor' *Journal of Medicinal Chemistry*, vol. 38, no. 14, July 7, 1995, pages 2774-2779.

I declare that the information, which is provided in the following paragraphs of this declaration, is a truthful description of results of experiments performed in the laboratories of Organon and filed as such in our archives.

The binding affinity of compounds for the estrogen receptors can be determined in an *in vitro* assay with recombinant Chinese hamster ovary (CHO) cells stably transfected with the human estrogen receptor  $\alpha$  (hER $\alpha$ ) or  $\beta$  (hER $\beta$ ). Competitive binding studies are performed using radiolabelled 17 $\beta$ -estradiol as a competitor ligand and the affinity of a test compound for the cytosolic estrogen receptors ER $\alpha$  and  $\beta$  is expressed as an IC<sub>50</sub> ratio relative to 17 $\beta$ -estradiol (100%).

The agonist and antagonistic transactivation activity of compounds on the estrogen receptors can be determined in an *in*

vitro assay with recombinant Chinese hamster ovary (CHO) cells stably co-transfected with the human estrogen receptor  $\alpha$  (hER $\alpha$ ) or  $\beta$  (hER $\beta$ ), the rat oxytocin promoter (RO) and the luciferase reporter gene (LUC). Agonist efficacy, i.e., the amount of maximal activation of the receptor by a compound, is expressed as fractional activation and compared to the efficacy of 17 $\beta$ -estradiol (100%). A more detailed description of the methodology can be found in De Gooyer M.E., Deckers G.H., Schoonen W.G.E.J., Verheul H.A.M. and Kloosterboer H.J., *Steroids*, Vol 68, pp21-30 (2003).

The following results, obtained by these methods, are shown in a revised version of Table A, page 14 of the subject application:

Table A

- A: Compound relative binding affinity at hER $\alpha$ .  
B: Compound relative efficacy at hER $\alpha$ .  
C: Compound relative binding affinity at hER $\beta$ .  
D: Compound relative efficacy at hER $\beta$ .

Compound	A	B	Conclusion	C	D	Conclusion	Rating
1	136	1.32	agonist	87	1.58	agonist	-
2	99	1.19	agonist	82	1.17	agonist	-
3	116	0.51	agonist	96	0.00	antagonist	+
4	93	1.31	agonist	169	1.66	agonist	-
5	95	1.09	agonist	87	0.33	antagonist	+
6	155	0.78	agonist	126	0.00	antagonist	+
7	43	1.08	agonist	96	0.96	agonist	-
8	104	0.70	agonist	127	0.00	antagonist	+
9	170	1.35	agonist	150	1.60	agonist	-
10	190	1.19	agonist	115	0.57	agonist	-
11	70	0.46	agonist	71	0.00	antagonist	+

From the results it can be concluded that all compounds (1-11) have good affinity for both estrogen receptor subtypes  $\alpha$  and  $\beta$  as is evidenced by their relatively high binding affinity for hER $\alpha$  (Table A, column A) and hER $\beta$  (Table A, column C).

From the results it can also be concluded that substantially similar compounds can produce different functional effects at both estrogen receptor subtypes  $\alpha$  and  $\beta$ . Thus whereas the 11 $\beta$ -butenyl derivative (compound 2) behaves as an agonist on ER $\alpha$  and an agonist on ER $\beta$ , the 11 $\beta$ -pentenyl homologue (compound 3) behaves as an agonist on ER $\alpha$  but as an antagonist on ER $\beta$ .

Similarly, whereas the 11 $\beta$ -butynyl derivative (compound 4) behaves as an agonist on ER $\alpha$  and an agonist on ER $\beta$ , the 11 $\beta$ -pentynyl derivatives (compounds 5 and 6) behave as agonists on ER $\alpha$  but as antagonists on ER $\beta$ .

The differential properties observed between the C4 and C5 11 $\beta$ -homologues, as demonstrated in the table of results, is unexpected and does not follow in any way from the teachings of either Lobaccaro *et al.* or Napolitano *et al.*

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 17 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Number of pages of this declaration: 5 pages.

22-07-2004

Date



A. G. H. Ederveen